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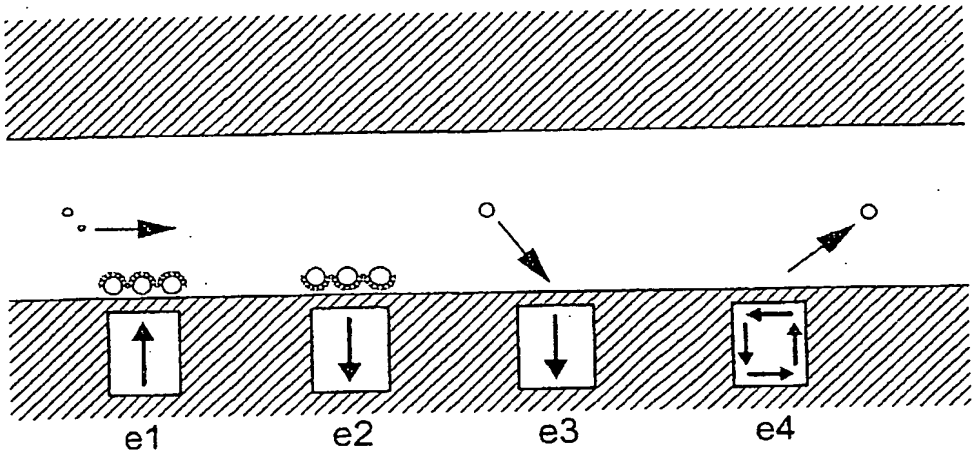
(54) Title: ARTIFICIAL STRUCTURES WITH MAGNETIC FUNCTIONALITY



(57) Abstract: A structure having magnetic functionality comprising at least one magnetic element having a fixed position, wherein said fixed element is a magnetic element, the magnetic polarity of which can be adjusted by application of an external magnetic field, and a second element being at least one movable separate magnetic element with an associated chemical or biochemical property. Preferably, said magnetic elements consist of a magnetic film deposited on a substrate in patterns, corresponding to the intended functionality of the structure, and in a size where each element of said pattern or patterns is large enough to exhibit a multi-domain magnetic microstructure. Preferably, said separate magnetic element is of a size where each particle is small enough to exhibit a mono-domain magnetic state, still large enough to exhibit a stable particle magnetic moment.

WO 03/043931 A1

Artificial structures with magnetic functionality.

The present invention concerns artificial biological and/or biochemical structures involving magnetic functionality, and in particular such structures having sub-micron or preferably nanometer resolution. The invention also concerns methods for the manufacture of such structures, their operation and use.

Background of the invention

The high efficiency of many biological structures and processes in nature is often the result of a high inherent flexibility of the structures or molecules involved. This is often combined with molecules, which are anchored or otherwise associated to surfaces in a three-dimensional network where the surface is one of the basic requirements for the ordered assembling of molecules achieved.

Nanotechnology plays a central role in the quest for creating artificial biologically functional surfaces. By using a combination of technologies, such as lithographic and scanning probe techniques, it is now possible to organise molecules onto surfaces at the nanometer scale. One method describing the formation of nano-scale defects with high resolution has been disclosed in WO 99/15895 by Oscarsson *et al.* The available techniques in themselves do however not suffice to mimic biological functions, as the flexibility at the molecular level – compared to the biological structure – is still missing, and it is yet not possible to regulate the processes as desired.

Prior art

The published international application WO 01/22088 discloses particles having a superparamagnetic core, and their use as therapeutic agents, carrying effector molecules chosen among cytostatics, toxins, enzymes, heavy metals etc. The particles are said to have a diameter of 10 nm or less, and their magnetic properties are used to direct the particle to a specific location. Their use as sub-components in a bioassay is briefly mentioned but not elaborated further. The application of said particles is mainly in the separation and/or concentration of desired components in a solution or reaction mixture.

Similarly, also WO 01/19405 discloses magnetic nanoparticles, and in particular magnetic nanoparticles, which can specifically bind to intracellular bio-macromolecules so that a

separation can be performed by the action of an external magnetic field. One application is the marking and separation and/or removal of malign cells as a step in the treatment of various forms of cancer.

Further, the published international application WO 01/11360 discloses a method for detecting binding reactions through measurement of the relaxation of birefringence of magnetic nanoparticles. Substances with structurally specific binding properties towards the analytes to be determined are labelled with ferri- or ferromagnetic substances, forming a magnetic complex or nanoparticle, and then added to the sample selected for analysis. After application of an external magnetic field, the relaxation of birefringence of the magnetic complexes is determined.

WO 97/46882 in turn discloses an apparatus and a method for separating, immobilizing, and quantifying biological substances from within a fluid medium by employing a high internal gradient magnetic capture structure formed within a vessel, in conjunction with an externally-applied force for transporting magnetically responsive material towards the capture structure.

Tanase M. *et al.*, "Magnetic Alignment of Fluorescent Nanowires" in Nano Letters, 2001, Vol. 1, No. 3, 155-158 disclose the orientation of nickel nanowires in magnetic fields.

Notably, the prior art seems to dwell on simple applications only, e.g. applications for separation, and fails to disclose anything but passive, permanent magnetic structures which are not suitable for the regulation / activation of biological complexes.

The objectives of the invention

One objective of the present invention is to create artificial biotechnological structures with a high degree of order while retaining a flexibility that makes it possible to manipulate the structure in four dimensions. In other words, a structure according to the invention should be operable not only in space, as in two or three dimensions, but also in time, e.g. activated or deactivated at a desired point in time.

Another objective is to create the means for investigating and developing the possible applications of, and exploring the limits of magnetic forces on a magnetic system in a liquid based environment. Another, closely related objective is to create the means for investigating and developing the possible applications of and the limits of magnetic forces on a magnetic system in a two- or three-dimensional solid or semisolid environment.

Another objective is to create tools for the investigation of the speed and forces involved in manipulating e.g. rotating magnetic particles provided with different kinds of molecules and cells, and to investigate the forces between individual macromolecules immobilised on different magnetic particles. One application is for example the determination of binding constants between individual macromolecules in a magnetic field.

Further, an objective of the present invention is to make available the means and methods for the investigation of the effects of generated electromagnetic fields on cells and biological reactions, such as bioaffinity reactions.

Further, an objective is to make available the means and methods for the investigation of molecular interactions in magnetic environments and/or by use of magnetic forces.

Yet another objective is to make available the means and methods for the investigation of fluidic dynamics in the sub-micron and nanometer ranges.

Yet another objective is to create functional components, which can find utility in nanomechanical applications, for example for controlled (with respect to location, movement, and time) manipulation of minute amounts of sample, analyte or even single molecules or particles.

Further objectives of the invention will be evident from the embodiments disclosed, either alone or in light of the advantages they present.

Summary of the invention

The present inventors have surprisingly shown that by using externally operable magnetic elements present on a surface or below or above the same, in combination with separate magnetic particles, chemical and biochemical processes can be finely regulated down to the nanometer scale. As the magnetic elements are externally operable, a particular function can be initiated at a particular location, and at a particular time, as desired. This invention makes available means and methods, which are of interest for biotechnological research, and make possible many biotechnological, medical, diagnostic as well as chemical applications. Applications of the present invention focus e.g. at the miniaturization of chemical and biochemical analysis and synthesis, the regulation of biotechnological processes, and the investigation of molecular/surface interactions in magnetic environments, which now can be controlled or optimised with a sub-micron or nanometer resolution.

Various advantages arise from the present invention. For example, the interaction between molecules, macromolecules, particles and/or cells can be regulated and/or determined with high precision. Devices according to the invention have many uses, for example in the fields of analysis, diagnosis, synthesis, and therapy. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims, which are hereby incorporated in their entirety.

Short description of the drawings

The invention will be disclosed in further detail in the following description, examples and drawings, in which

Fig. 1 shows schematically how a magnetic microstructure consisting of a magnetic element 1 is used to manipulate magnetic particles 2 having particular surface properties, here illustrated by immobilised groups 3, thus achieving a mechanism where these properties are either displayed as shown in (b), or hidden as shown in (c) as desired. The arrow "H" illustrates the external magnetic field. The arrows drawn in the elements show the magnetic polarity of the elements.

Fig. 2 shows schematically how magnetic particles 2 are used to move macromolecules on a surface, for example between different magnetic elements 4, 5, and 6, arranged on the surface. The external magnetic field is indicated with an arrow H, first pointing right, then down (H′)

Fig. 3 illustrates how magnetic elements can be supplemented by geometric features on a non-magnetic material covering the magnetic elements. Such features are used to influence the orientation or location of magnetic particles drawn to said elements, alternatively to modify the influence of the magnetic element on its surroundings, here illustrated by the flow channel formed between layers III and IV. In (a) the element e1 is covered by a non-magnetic layer, exhibiting a locally increased thickness, a local hillock or raised area above the element; in (b) the non-magnetic is thinner or exhibits a hole, locally above element e2; in (c) the non-magnetic layer is thicker in an area, larger than the size of the underlying magnetic element e3. The layers I, II, III, and IV, illustrate a substrate and non-magnetic layers respectively, defining the geometry of the device in which the elements are incorporated. Layers I, II, III, and IV can be manufactured of the same or different materials, preferably a thermoplastic material.

Fig. 4 illustrates how features of the substrate can be utilised to influence the deposition of the magnetic film and thus to form magnetic elements of different configuration, as well as discontinuities in an otherwise magnetic surface. "s" denotes a substrate, and "m" denotes the magnetic layer. The substrate s can consist of several layers (not shown).

Fig. 5 illustrates schematically how magnetic elements positioned along a flow channel can be used to influence components present in a flow, passing said elements, e.g. a liquid flow in a flow channel defined by layers of non-magnetic material. From left to right, the element e1 is activated in order to make the particles magnetically immobilised thereto expose their functional groups; element e2 is given an opposite polarity in order to make similar particles hide their functional groups; element e3 is activated in order to capture magnetic particles from the flow of liquid; and e4 is deactivated in order to release previously captured particles.

Fig. 6 illustrates in (a) how one or more magnetic particles can be used to stir a liquid in a miniature flow cell, and (b) how a magnetic particle can be used to create a miniature valve, directing the flow in small passages. By externally activating or by externally changing the polarity of a magnetic element A, positioned within effective reach of the particles, e.g. below, above, or alongside the flow cell, the movement of a particle or particles p is controlled. The movement of the particle, from a "closed" to an "opened" postion of the valve, and vice versa, is achieved by one or more magnetic elements, here shown as B and C, positioned alongside, below or above the passage. When the magnetic element B is activated, using an external magnetic field, and its polarity properly adjusted, the particle p is immobilised adjacent to the wall of the passage, next to B, and the valve kept open. When B is inactivated, or its polarity reversed, and C correspondingly activated, and its polarity properly adjusted, the particle p is drawn to a position where it closes the valve.

Fig. 7 a illustrates schematically how a magnetic particle with a particular property, such as hydrophobicity / hydrophilicity or the presence / absence of a macromolecule, such as a particular ligand, can be rotated within a small chamber, and e.g. used to transport minute amounts of a substance from one compartment to another in miniaturized flow systems. The position of the particle is operated using one or more magnetic elements (not shown), activated / deactivated by corresponding external magnetic fields. The particle is contained in a constriction in a channel, said constriction defining a compartment holding the particle, but not restricting its movements. Fig. 7 b shows a perspective view of such constriction.

Description

In the below description, examples and claims, the following terms will be used and are here attributed a specific meaning:

The prefix "nano" meaning one billionth (1/1,000,000,000) has here been used in combination with various nouns, defining their properties, as in the word "nanoparticle". This term is used for describing particles having an average diameter of 100 nm or less. In a similar manner, the term "nanostructure" and "nanometer resolution" defines structures having features having an average size of 100 nm or less in at least one dimension.

The term "pattern" is used to define an ordered distribution of features, e.g. features of a structure or features present on a surface, in contrast to random or unordered features. In this context, the term micron resolution defines structures of size approximately 1 micrometer, while sub-micron resolution defines a structure having an average diameter in the interval 0.1 to 1 micrometer.

The term "array" also defines an ordered distribution of features, in contrast to random or unordered features. In an array, each feature is identified by its location, its coordinates within the array. Normally arrays are patterns arranged in rows and columns, both also other formats can be envisioned, provided that the unique coordinates of each feature can be identified.

The term "chemical or biochemical property" is used to define properties such as affinity or repulsion, binding properties, hydrophilic / hydrophobic properties, specific reactions between chemical entities, between a particle and a chemical entity, between a particle and a cell, between cells, between chemical entities and cells, such as the reaction between enzyme and substrate, between catalysts and reactants, between enzymes and cofactors, between antibody and antigen etc.

The term "chemical or biochemical reactions" meant to encompass all chemical and biochemical reactions where it is desirable to regulate or enhance the behaviour of components on or in the vicinity of surfaces, and in particular minute amounts of such components, such as substrates, analytes, ligands, reagents, receptors, cofactors etc. in chemical and biochemical synthesis, analysis, assays etc. In this context, the term receptor comprises all types of receptors, such as membrane bound receptors, soluble receptors, lectins, antibodies, fragments of antibodies, peptides, and synthetic receptor molecules.

The term "analyte" is used in a broad sense of the word, encompassing any chemical entity to be handled or determined for chemical, biochemical, diagnostic or therapeutic purposes.

The term "reaction vessel" when used in this context means any vessel capable of containing a reaction mixture of interest. Examples of reaction vessels suitable for use according to the invention include, but are not limited to, the following: test tubes, so called micro tubes, Eppendorf-tubes, a single well or a multitude of wells in a microtitre plate, such as a microtitre plate of the 96-hole format, and various formats with a high density arrays of wells, such as the 192-hole format, the 384-hole format, denser formats or the like. One aspect of the invention is the production of functional components, which can be applied in such reaction vessels, or integrated therein. A preferred embodiment of the invention is however the application of the invention to miniaturized reaction vessels, including flow cells, flow channels, cells and volumes, defined in platforms and devices of various formats, such as a disc or other device, e.g. the analysis platform corresponding to the conventional CD-format.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials for the practice or testing of the present invention are described below, other methods and materials similar or equivalent to those described herein, which are well known in the art, can also be used. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The present inventors here make available a three-dimensional structure having micron resolution, and preferably sub-micron resolution and magnetic functionality, preferably with a nanometer resolution, wherein said structure comprises a first element being a substrate having a magnetic film deposited thereon, and a second element being at least one separate magnetic element with an associated chemical or biochemical property. Said first element is a magnetic element, e.g. a thin magnetic film deposited on an otherwise non-magnetic substrate. According to the invention, said first magnetic element is externally operable, meaning that it can be activated or deactivated, and its magnetic polarity adjusted as desired, by application of an external magnetic field.

Suitable magnetic materials include soft magnetic materials, such as Fe, Fe-Si alloys, , Ni-Fe alloys, Ni-Fe-Cu-Cr alloys etc. Preliminary experiments indicate that the thickness of the magnetic film or magnetic element preferably is in the interval of 20 to 50 nm.

Importantly, said magnetic element is of a size which guarantees that said element in zero external field comprises at least four magnetic domains. According to one embodiment, said element is a rectangular element comprising four magnetic domains, or a higher amount of magnetic domains, however an amount of domains forming a closed magnetic circuit when the element is not exposed to an external magnetic field. The geometry of the element may be varied, that is the element can have other shapes than rectangular. Rectangular in this context also comprises bar shaped elements, embedded into a substrate, or protruding therefrom, exposing either their long side or short side to the surface. Exposing can also mean that the short or long side of the element is present slightly below the surface, or slightly above, in the inverted case, where the surface in question e.g. defines the ceiling of a channel or compartment.

The shape of the element can, according to the invention, be used to influence the formation of magnetic domains. The magnetic element can be square, rectangular, circular, elliptic. The magnetic element can surprisingly also be formed as a discontinuity in a magnetic film, e.g. a hole in a magnetic film, deposited on an otherwise non-magnetic substrate. The magnetic elements can also be shaped as a ring or donut, as well as the corresponding negative images thereof. A magnetic element can also be shaped as a raised or lowered subsection in a substrate. It is also conceived, and constitutes an aspect of the invention, that the magnetic element may be present in combination with a non-magnetic structure, which canalises or otherwise orientates the particles. In other words, a discontinuity can be used to define a magnetic element.

Embodiments of this particular aspect of the invention comprises non-magnetic layers superimposed on the magnetic elements, said layers having features, such as depressions, rises, ridges, etc which aid in the positioning and optionally determine he orientation of the magnetic particles. See Fig. 3.

One particular embodiment of the invention involves circular magnetic elements. In such elements, the magnetic polarity in the centre of the circular elements can perpendicular to the plane, either directed inwards, into the plane, or outwards, out of the plane. This can have particular applicability for the orientation of magnetic particles, either alone, or in combination with non-magnetic structures and geometrical features, superimposed over the magnetic element.

According to the present invention, the second element is a magnetic particle, that is a particle small enough to exhibit a mono-domain magnetic state, but still large enough to exhibit a stable particle magnetic moment, and preferably of a size in the interval of less than about 500 nm, preferably less than 300 nm and most preferably less than 100 nm. A size interval of about 20 to about 50 nm is currently held to be most preferable. Said particle preferably has a magnetic core covered by a non-magnetic shell, e.g. a dextran shell, which aids in the immobilisation of functional groups etc to the particle.

According to one aspect of the present invention, the magnetic particles are provided with an external property, other than their magnetic property. See particle 2 in Fig. 1. This can consist of different physical or chemical properties of the surface of the particle, such as its reflectance, absorption, adhesion properties, affinity for a particular chemical species, hydrophobicity / hydrophilicity, immobilised macromolecules such as antibodies, substrates, enzymes, co-factors, catalytically active components, etc. Preferably said particles have one of the above properties on only one hemisphere, the other hemisphere exhibiting another property, or at least the absence of the property on the opposing hemisphere. Preferably said hemispheres exhibiting different properties are oriented in correspondence to the magnetic polarity of the particle. Using an analogy, the "north" hemispheres has one property over its surface, whereas the "south" hemisphere lacks this property or exhibits a different property over its surface.

Such particles in them selves constitute an embodiment of the invention. One aspect of the invention is a method for their production outlined in the following:

Single domain, stable magnetic cores are given a shell suitable for the desired treatment. For example, for physical modifications, a shell of a thermoplastic resin is suitable. The particles are then evenly distributed on surface and both oriented with the aid of a magnetic field. When in the desired position, e.g. exhibiting their "north" hemispheres, but hiding their south hemispheres, the particles are subjected to a treatment resulting in the desired property being localised to the exposed hemisphere only. For example, by exposing polystyrene particles to UV radiation, the hydrophobic properties can be influenced. Another example, when one desires to influence the reflectance properties of the particles, involves immobilising the particles as outlined above, whereupon a thin metallic layer is sputtered or evaported on the exposed hemisphere of the particles.

Preferably said first element consists of a substrate having magnetic features exhibiting micron or sub-micron resolution, such as a magnetic or magnetizable film applied in an ordered pattern on a surface. Fig. 4 shows an example where a magnetic film has been applied to a patterned surface, shown in cross section. The surface pattern can be designed so, that the transition between higher and lower parts is very steep. As a consequence of this, the layer of magnetic material will be unevenly deposited when e.g. sputtered or evaporated onto the surface. The magnetic material layer will be thinner at the slanted portions, and thicker at the horizontal portions. As material can be evenly removed, e.g. by etching, this means that all material can be removed from the slanted portions, leaving a magnetic layer only at the horizontal area. This makes it possible to create said features, discontinuities etc mentioned above.

The present invention also makes available a method for the manufacture of sub-micron three-dimensional structures comprising a substrate and means having magnetic functionality, wherein a magnetic film is arranged on the substrate in a pattern with sub-micron resolution, said pattern corresponding to the intended functionality of the structure, and magnetic particles are coupled to chemically functional groups, and brought in contact with said pattern on said substrate.

The present invention further makes available a method for operating sub-micron functional means including a structure as disclosed in the description and examples, wherein an external magnetic field is applied to the structure in order to alter the direction of the magnetic moment of at least one of the elements of the pattern of the surface, or in order to manipulate the position, orientation or movement of at least one of the magnetic particles.

A nanostructured magnetic pattern can for instance consist of bar shaped soft magnetic elements of e.g. permalloy of size a few tenths of a micron, separated from each other by a distance, e.g. a distance in the range 0.1 – 1 μm. The size of each magnetic element is large enough to exhibit a multi-domain magnetic microstructure. Moreover, in zero applied field, the magnetic microstructure should correspond to that of a closed magnetic structure, that is the magnetizations in different domains should in zero field form a closed magnetic circuit (Fig. 1 (a)).

A magnetic colloid will be used to attach macromolecules to the surface of magnetic particles, preferably nanoparticles of size a few tens of a nanometer. The size of each nanoparticle is

small enough to exhibit a mono-domain magnetic state, still large enough to exhibit a stable particle magnetic moment, meaning that superparamagnetic relaxation should be negligible. Adding this (magnetic) solution to the nanostructured surface, the magnetic nanoparticles will be attracted to the poles of the bar shaped magnets, cf. Fig. 1 (b) where an applied magnetic field has magnetised the elements to saturation, thereby immobilizing the macromolecules (magnetic nanoparticles). It is also possible to rotate the particles, thereby turning a process on or off, transporting or transposing bound particles, cells or macromolecules by changing the direction of the applied field (cf. Fig. 1 (c)).

Embodiments of the invention consequently comprise means and methods for exposing and manipulating cells, particles and macromolecules. Using one or more magnetic particles attached to a macromolecule, the orientation of the macromolecule can be regulated by magnetic forces, e.g. in order to expose different parts, such as active sites, binding sites etc of the macromolecule. Accordingly, a macromolecule such as a polypeptide or an oligonucleotide can be stretched, contracted, folded or straightened at will.

It is also desirable to be able to move macromolecules on the nanostructured surface between different elements of the surface. This may for instance be accomplished by rotating the applied magnetic field, as indicated in Fig. 2.

According to one embodiment of the invention, a series of magnetic elements are arranged on a surface, defining a path for transporting magnetic particles. The magnetic elements can be individually activated by application of an external magnetic field. The elements may possess three different states; left-hand polarity, right-hand polarity or a neutral, non-polarized state. By individually activating the elements, and by changing their polarity, magnetic particles can be transported along the path formed by said elements. In an embodiment comprising three immobilised elements, when only the first has been activated, and given a right-hand polarity, particles are drawn to the left end of said element. When also the second element has been activated, and the polarity of the first element inverted (if necessary), the particles are drawn to the gap between said first and second element. When the first element has been deactivated, and the polarity of the second element inverted, the particles are relocated to both ends of said second element. When the third element has been activated, and the polarity of the second element inverted (if necessary) the particles are drawn to the gap between said second and third element. When finally only the third element is activated, and its polarity inverted, the particles are drawn to both ends of said element.

According to another embodiment of the invention, illustrated in Fig. 3, features of a non-magnetic material, superimposed on the magnetic elements, is used to direct, e.g. to focus the particles to the elements. In Fig. 3, e1, e2 and e3 denote separate magnetic elements on a substrate I. The magnetic elements are separated by a non-magnetic material II. Further, the magnetic elements are covered by a second non-magnetic material III. Naturally n1 and n2 can be the same. In a, said non-magnetic material forms a discrete hill above the magnetic element e1. In b, a hole is provided in the non-magnetic material, right above the element e2, and in c, a raised surface is provided above the element e3.

In Fig. 4 it is shown how the magnetic elements can be formed on a non-magnetic substrate having a geometry which influences the application of the magnetic film, e.g. when such film is sputtered on a surface, followed by etching to remove the materials in particular areas, relying on the phenomena that the sputtered film is deposited mainly on the flat surfaces, and that the film adhering to the slanted surfaces can be removed by etching, leaving a sufficient thickness of film in the plane areas.

Fig. 5 illustrates an embodiment where magnetic elements e1, e2, e3, and e4 are placed in association to a flow channel. Elements e1 and e2 both engage magnetic particles having a particular property or a functional group on one hemisphere, and another property or the absence of said property on the opposite hemisphere. By activating e1 and adjusting the polarity, the magnetic particles are made to expose their property, e.g. exposing functional groups interacting with a component in the flow of samples etc passing in the flow channel. Regarding element e2, its polarity has been adjusted so, that the magnetic particles are hiding their functional group or particular property, and thus unable to interact with components in the flow. Element e3 has been activated, and its polarity adjusted so, that it attracts magnetic particles present in the flow. Conversely, element e4 has been inactivated, so that it releases magnetic particles formerly immobilised on its surface. In Fig. 5, the magnetic elements have been shown as arranged at the "bottom" of the flow channel. It is however understood that the flow channel can be freely rotated, and the magnetic elements thus positioned above, or alongside the flow.

Fig. 6 illustrates two embodiments of the invention, (a) showing how one or more magnetic particles can be used to stir a liquid in a miniature flow cell, and (b) how a magnetic particle can be used to create a miniature valve, directing the flow in small passages. The external

magnetic field operating the particles can be created in different ways, for example using miniature solenoids placed around, below, above or to the side of the flow cell or the passage.

In Fig. 6a a magnetic element A is shown as being positioned below or alongside (dashed lines) the flow cell. By alternatingly activating and deactivating the magnetic element A, or by changing the polarity of the same, using an external magnetic field, the particle or particles are moved back and forth in the cell.

In Fig. 6b two magnetic elements B and C are shown, positioned above or below the valve. When the magnetic element B is activated, using an external magnetic field, and its polarity properly adjusted, the particle p becomes temporarily immobilised adjacent to the wall of the passage, next to B, and the valve kept open. When B is inactivated, or its polarity reversed, and the magnetic element C correspondingly activated, and its polarity properly adjusted, the particle p is drawn to a position where it closes the valve.

Fig. 7a illustrates schematically an embodiment, where a magnetic particle is localized between two defined volumes, e.g. two compartments in a miniaturized analysis platform. Below, above, or otherwise positioned so that it can influence the orientation of said magnetic particle, is a magnetic element (not shown). The magnetic particle preferably has one functionality, such as hydrophobical properties or one type of functional groups associated with or attached to one hemisphere, and another functionality / other groups, or the absence of such functionality or groups at the opposite hemisphere. By changing the polarity of the magnetic element, the orientation of the magnetic particle is influenced. A magnetic particle carrying receptors for a particular compound can be used to detect the presence of said compound in one of the compartments to which a sample is led, and then recharged in the opposite compartment, containing e.g. a buffer solution or rinse solution where said compound is absent. The adhesion of the compound to be determined to the magnetic particle can be detected by observing changes in movement, rotation or magnetic responsiveness of said magnetic particle.

Fig. 7b shows a perspective view of the above embodiment.

Further embodiments of the invention, as well as brief outlines as to their manufacture will be given below:

Structured magnetic surfaces can be prepared using different types of lithography. For structures down to micrometer level photolithography is used. A photoresist is spinned onto the surface, after which the surface is illuminated through a mask (prepared in advance using a CAD program and mask generator) containing the structures of interest. The resist is developed, removing the exposed parts. Next, magnetic or magnetizable material can be evaporated or sputtered onto the surface before lifting off the remaining resist.

Similarly, to obtain structures with nanometer dimensions (down to 5 nm with a 200 kV, and 30 nm with a 40 kV accelerating voltage), fine patterns can be written, using the electronbeam of a Scanning Electron Microscope (SEM) into a polymer resist that is spinned onto the surface. Patterns are designed in a CAD program, after which the external PC simultaneously controls the x-y scan coils and beam blanker of the SEM through an interface. Exposure doses can be adjusted by varying the exposure time and/beam current, and are chosen to break the polymer bonds (positive resist, or to crosslink them at higher dose, negative resist). Samples are developed in order to remove the exposed resist, after which the desired magnetic or magnetizable material can be deposited by evaporation/sputtering. Subsequent lift-off will result in a surface with sub-micron or even nanometersized structures of the evaporated material.

Furthermore, several techniques collectively known as 'soft lithography' can be used to pattern surfaces. They all employ stamps, which are prepared by casting pre-polymer against a master produced by conventional lithography. A non-exhaustive list of suitable methods, applicable to the manufacture of magnetic surfaces and patterned magnetic surfaces according to the invention include the following methods:

Near-Field Phase Shift Lithography: A transparent polydimethylene siloxane (PDMS) phase mask with relief on its surface is placed in conformal contact with a layer of photoresist. Light passing through the stamp is modulated in the near-field. If the relief on the surface of the stamp shifts the phase of light by an odd multiple of π, a node in the intensity is produced. Magnetic features with dimensions between 40 and 100 nm are produced in photoresist at each phase edge.

Replica Molding: A PDMS stamp is cast against a conventionally patterned master. Polyurethane is then molded against the secondary PDMS master. In this way, multiple copies

can be made without damaging the original master. The technique can replicate features as small as 30 nm.

Micromolding in Capillaries (MIMIC): Continuous channels are formed when a PDMS stamp is brought into conformal contact with a solid substrate. Capillary action fills the channels with a polymer precursor. The polymer is cured and the stamp is removed. MIMIC is able to generate features down to 1 μm in size.

Microtransfer Molding: A PDMS stamp is filled with a prepolymer or ceramic precursor having the desired magnetic or magnetizable properties and placed on a substrate. The material is cured and the stamp is removed. The technique generates features as small as 250 nm and is able to generate multilayer systems.

Solvent-assisted Microcontact Molding: A small amount of solvent is spread on a patterned PDMS stamp and the stamp is placed on a polymer, such as photoresist. The solvent swells the polymer and causes it to expand to fill the surface relief of the stamp. Features as small as 60 nm have been produced.

Microcontact Printing: An "ink" of alkanethiols is spread on a patterned PDMS stamp. The stamp is then brought into contact with the substrate, which can range from coinage metals to oxide layers. The thiol ink is transferred to the substrate where it forms a self-assembled monolayer that can act as a resist against etching. Features as small as 300 nm have been made in this way.

One practical method of creating discrete magnetic elements on a surface, displaying high resolution, is based on the contact moulding of thermoplastic surfaces against a master copy. The resulting surface, displaying a pattern of raised and depressed areas, is then subjected to sputtering, whereby a layer of magnetic material is deposited on the surface. Surplus material is removed by chemical etching, leaving the desired pattern of discrete magnetic elements. It is conceived that the magnetic elements can be covered in further layers of thermoplastic, the layers defining a desired geometry, such as compartments, flow channels etc. Such compartments and flow channels can then be given desired chemical properties, for example by including reagents etc in said compartments / channels. Presently, this is a preferred method for manufacturing miniaturized platforms for chemical and biochemical analysis.

According to one embodiment of the invention, magnetic nanometer sized beads or bars are partly derivatised, that is functional molecules or moieties are added only to a limited area of the bead or bar, for example to one hemisphere of a bead or to half the circumference of a cylindrical bar. This partial derivatisation is done by using evaporisation of different metals (e.g. gold) or chemicals (e.g. silanols) on beads or bar shaped particles when they are immobilised (e.g magnetically) to a surface. This makes it possible to immobilise different molecules with different chemical characteristics to different regions of the magnetic particles. The final result can for example be that one hemisphere of a bead is coated with hydrophobic molecules, the opposite side is hydrophilic or one side of a molecule is coated with one kind of cell/biomolecule/molecule, and the opposite side is coated with another kind of cell/biomolecule/molecule. According to this, catalytic parts for chemical reactions can be immobilised on one side of the magnetic particles and inhibitors or "nothing" on the other side. Cells could be immobilised on one side, which will make it possible to move the cells, e.g. from one compartment to another for regulation of activity.

All these alternatives make it possible to regulate an activity by time and change the surface characteristics by time for exposure to bulk solutions.

Since the magnetic particles are possible to immobilise via magnetism an alternative approach could be to approach the beads on the magnetic film close to a liquid for derivatisation by use of a piezocrystal. The same technique could be useful for transfer of magnetic set-ups from one magnetic film to another for transfer of biological information from one surface to another.

One aspect of the invention is its use in applications relating to fluid dynamics. According to one embodiment, the flow of liquid in micro- or nano-fluidic systems, is regulated by use of magnetic beads operated using magnetic elements, activated by external magnetic fields. The beads will serve as a "tap" at constrictions of the fluidic system forming controllable valves which make it possible to regulate liquid flow also in very small scale devices where regulation of the movement, dispensing or flow of chemicals or biological molecules is of importance. Particular embodiments include, but are not limited to drug release systems for *in vivo* or *in situ* use, regulators of devices dispensing reagents for chemical reactions. Magnetic beads according to the present invention can also be used for stirring liquids in small compartments, for closing liquid/surface interfaces or for moving the beads close to the surface of tubes for cleaning procedures.

According to one embodiment of the invention, one or more magnetic beads is/are provided with antibodies and placed in a small constriction or compartment where at least one bead can be subjected to an analyte and made to move, rotate, oscillate or vibrate in said constriction. The movement, rotation or the like will continue as long as there is no biospecific reaction between the analyte and the antibody. Changes in movement, e.g. rotation, are then detected and taken as an indication that the antibody has reacted with its counterpart.

When such a magnetic bead is placed in a limited space or constriction connecting two compartments, it becomes possible to transport molecules or cells one by one from one compartment to another by rotating the magnetic bead. The size of the constriction and the beads will limit the number of cells or molecules actively transported and the selection of binding moieties affixed to the beads determines with high selectivity which cells or molecules are transported. An example is illustrated in Fig. 7a and 7b.

Another aspect of the present invention is the application of the invention to catalytic systems. Molecules of different molecular weights are possible to immobilise to magnetic colloids such as porphyrin molecules and analogs useful for metal organic catalysis of industrially important processes. Such heterogenous organic catalysis make it possible to let a reaction take place with the catalysator immobilised to the nanomagnetic beads in a bulk solution and to regulate, e.g. increase the molecular transportation by stirring the catalysator during the reaction, and finally to attract the particles e.g. to the magnetic elements of a surface which separate the catalytic unit from the products in an efficient way.

Further, the invention can be used to regulate ON/OFF mechanisms for chemical reactions where catalyst molecules are involved. In such systems the catalytic unit is positioned on one side of the hemisphere of a nanometer sized magnetic bead or on one side of a bar shaped magnetic particle, preferably a nanoparticle. By rotating the magnetic particles the catalyst will be exposed to the bulk solution or be sterically hindered from reaction.

Yet another aspect of the present invention is the application of the invention to biocatalytic systems. As biocatalytic systems often are regulated by cofactors, the present invention makes it possible to regulate biochemical reactions with high accuracy and precision by moving magnetic particles immobilised with enzymes into compartments provided with the necessary cofactors.

Another aspect of the present invention is the application of the invention to hydrophobic/hydrophilic surfaces or to ion exchange surfaces. By use of magnetic beads or bars partly derivatised with one kind of molecule on one side and another one on the opposite side the possibility exist to create a surface by which e.g. the property of said surface could be changed by rotating the magnetic particles on the surface. Applying this principle, different gradients of hydrophobicity can be created for a surface. Alternatively one side of the surface can be an ion exchanger. These kinds of surfaces are commonly used for molecular separation, which makes it possible to achieve two different separation principles using one and the same surface.

Another aspect of the invention is the application of the inventive technology to applications where the desired functionality / property is one of absorption, reflection, or transmission of infrared, visible or ultraviolet radiation. Since the magnetic particles in the form of magnetic bars are possible to rotate at will, the efficiency of molecules absorbing incident light or *vice versa* can be regulated. This can be used e.g. for regulating the sensitivity or efficiency of solar cells.

Yet another aspect of the present invention is the handling of cells in magnetic environments. Cells in this context means both eukaryotic and prokaryotic cells, including organelles and cell fragments. Also viral particles and their fragments are included. The present invention makes it possible to organise and directly distribute or spread e.g. cells on surfaces by using cell growth stimulating factors immobilised to magnetic beads. One important embodiment is the stimulation of nerve cell growth on magnetic surfaces.

Further, immobilisation of cells to magnetic beads of nanometer dimensions makes it possible to change the position of cells and in this way regulate their activity by moving, adjusting or removing the magnetic particles.

One aspect of the invention concerns biomolecular motors. The possibility to position and move magnetic beads on surfaces, said beads having biomolecules immobilised onto them, makes it possible to create new artificial biological and biomechanical structures, as well as to investigate the functions of such structures. One embodiment is the creation of artificial respiratory chains or enzyme cascades, e.g. for generation of proton gradients or voltage.

Another aspect of the invention relates to the creation of ordered arrays of molecules, particles, cells or organelles on a surface. One example is the creation of nucleic acid and

protein arrays. Within the scope of the present invention, it is contemplated that magnetic particles are derivatised with a component having affinity to the chosen molecules, particles, cells or organelles that are to be arrayed with different kinds of antibodies/DNA templates. A first array of magnetic elements is then arranged on or within a surface. Said array of magnetic elements is then magnetised at sitespecific areas, that is one or several of the magnetic elements is/are magnetised as desired. Magnetic particles with one kind of antibody will then be directed to the desired positions and the procedure repeated for other sets of magnetic particles derivatised with other molecules, particles, cells or organelles. By further repeating this procedure, magnetic particles carrying different molecules, particles, cells or organelles will be site specifically positioned on the surface in the form of an ordered array.

Further, as one embodiment of the invention, the array can have "built in" functionalities, for example in that a sub-group of the particles immobilised on the magnetic elements on the surface can be manipulated. By changing the polarity of selected magnetic elements, chosen particles can be repelled from the surface, or their orientation changed as desired. One specific embodiment is a flexible ordered array, i.e. an ordered array, in which individual locations or groups of locations can be addressed, and their properties manipulated. Addressed and manipulated in this context means not only that molecules, particles, cells or organelles can be immobilised at specific locations or coordinates as desired, but also that they can be turned in their positions, activated or deactivated or released or even repelled from their positions as desired.

Immunocomplexes formed in solution outside the surface together with a marker molecule are then added to the surface and immobilised biospecifically via the antibody. Washing the surface eliminates the possible excess of marker. The local position of the marker will then give information as to what kind of analyte was present in the sample.

Another aspect of the present invention is the possibility to produce novel diagnostic devices and instruments for research and analysis. The present invention makes available the methods and devices for the construction of diagnostic devices. One embodiment of the invention is a device for the determination of blood glucose levels, preferably combined with glucose oxidase for detection and regulation of the concentration of sugar in blood.

Examples

In an experimental set-up, an array of 500.000 magnetic elements was constructed on a silicon substrate. The 500.000 magnetic elements were evenly distributed in rows and columns over an area of 2 x 2 mm. When applying an external magnetic field, an even activation of the elements was observed. When applying magnetic particles, preferably magnetic nanoparticles, to this array of magnetic elements, an even immobilisation of said particles on said area was achieved.

In laboratory experiments, a resolution of 20 to 50 nm has been achieved, but it is conceived that a finer resolution could be obtained when the methods are improved.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention as set forth in the claims appended hereto.

Claims

1. A structure having magnetic functionality comprising at least one magnetic element having a fixed position, and at least one mobile magnetic particle, **characterized** in that said fixed element is a magnetic element, the magnetic polarity of which can be adjusted by application of an external magnetic field, and a second element being at least one separate magnetic element with an associated chemical or biochemical property.

2. A structure according to claim 1, wherein said at least one fixed magnetic element is/are deposited on a substrate in a pattern of separate magnetic elements, each element being large enough to exhibit a multi-domain magnetic microstructure.

3. A structure according to claim 2, wherein said multi-domain magnetic microstructure corresponds to that of a closed magnetic structure, where the magnetizations in different domains in zero field form a closed magnetic circuit.

4. A structure according to claim 2, wherein said pattern of magnetic elements exhibits millimeter-resolution or preferably micron-resolution, and most preferably sub-micron resolution.

5. A structure according to claim 1, wherein said mobile magnetic particle comprises a magnetic particle exhibiting a mono-domain magnetic state and a stable magnetic moment or negligible superparamagnetic relaxation.

6. A structure according to claim 5, wherein said mobile magnetic particle is a nanoparticle.

7. A structure according to claim 1, wherein the associated chemical or biochemical property of the particle is one of reflectance, absorption, adhesion properties, affinity for a particular chemical species, hydrophobicity / hydrophilicity, immobilised macromolecules such as antibodies, substrates, enzymes, co-factors, catalytically active components or combinations thereof.

8. A structure according to claim 7, wherein the associated chemical or biochemical property is present at one hemisphere of the particle only, the other hemisphere having another or the opposite property.

9. A structure according to any one of the claims above, wherein the magnetic elements having a fixed position are present in the form of an ordered array, each element having defined coordinates, and the mobile magnetic particles directed to, manipulated on or repelled from said array by the application of external magnetic fields directed to one or more of said defined coordinates.

10. A method for the manufacture of structures comprising a substrate and separate means having magnetic functionality, **characterized** in that a magnetic film is arranged on the substrate in a pattern, said pattern corresponding to the intended functionality of the structure, and magnetic particles are coupled to chemically functional groups, and brought in contact with said pattern on said substrate.

11. A method for operating sub-micron functional means including a structure according to any on of claims 1 – 7, **characterized** in that an external magnetic field is applied to at least one fixed element changing its magnetic properties and influencing the movement or position of movable magnetic particles in the vicinity of said element.

12. A method for performing a chemical reaction, **characterized** in that the reaction is performed in an environment where at least one component taking part in the reaction is immobilised to a magnetic particle exhibiting a mono-domain magnetic state and a stable magnetic moment or negligible superparamagnetic relaxation, the movement, position or orientation of said particle being influenced by a fixed magnetic element, the magnetic polarity of which can be adjusted by application of an external magnetic field, and where an external magnetic field is applied to manipulate said magnetic particles in said environment.

13. A method according to claim 12, wherein the chemical reaction involves the interaction of substrates, analytes, ligands, reagents, receptors, cofactors in a chemical or biochemical synthesis, analysis, or assay.

14. A method for creating an ordered array on a surface, **characterized** in that magnetic elements are arranged on fixed positions on a surface in the form of an ordered array, each element having defined coordinates, whereafter mobile magnetic particles are directed to, manipulated on or repelled from said array by the application of external magnetic fields directed to one or more of said defined coordinates.

15. A device for regulating the contact time between two chemical entities, such as between a reagent and a sample, **characterized** in that said device comprises a structure according to claim 1, where one of the chemical entities is coupled to at least one movable magnetic particle.

16. A device for stirring liquids in small volumes, **characterized** in that said device comprises a structure according to claim 1, where at least one movable magnetic particle is/are put in motion by the application of an external magnetic field to a fixed magnetic element in the vicinity of said particle/-s.

17. A device for the transport of chemical or biological entities, macromolecules, particles, cells or organelles, **characterized** in that said device comprises a structure according to claim 1, where at least one movable magnetic particle exhibits specific affinity to the chemical or biological entity, macromolecule, type of particle or cell or organelle to be transported, and the movement of said at least one magnetic particle is regulated by the application of an external magnetic field to a fixed magnetic element in the vicinity of said particle/-s.

18. A device for regulating chemical and/or biochemical reactions, **characterized** in that said device comprises a structure according to claim 1, and that an effective molecule or molecules is/are coupled to one hemisphere of at least one movable magnetic particle, and the presentation of said molecule/-s is/are regulated through the application of an external magnetic field to a fixed magnetic element in the vicinity of said particle/-s thus influencing the orientation of said particle/-s.

19. A device for detecting an analyte in minute amounts, **characterized** in that said device comprises a structure according to claim 1, and that a compound with specific affinity to said analyte is coupled to at least one movable magnetic particle, and where a change in the behaviour of said particle is taken as an indication that a reaction between the analyte and said element has taken place.

20. A device for the distribution of individual cells on surfaces, **characterized** in that said device comprises a structure according to claim 1, and where cell growth stimulating factors are immobilised to the movable magnetic particles.

21. A miniature valve comprising a valve seat and a particle, fitting in said valve seat and closing said valve, **characterized** in that said particle is a magnetic particle exhibiting a mono-domain magnetic state and a stable magnetic moment or negligible superparamagnetic relaxation, and that said particle is drawn to said valve seat by a magnetic element, the magnetic polarity of which can be adjusted by application of an external magnetic field.

22. An ordered array of at least one component on a surface, **characterized** in magnetic elements having a fixed position are present in the form of an ordered array, each element having defined coordinates, and mobile magnetic particles carrying said at least one component are associated with said elements, and manipulated by the application of external magnetic fields directed to one or more of said defined coordinates.

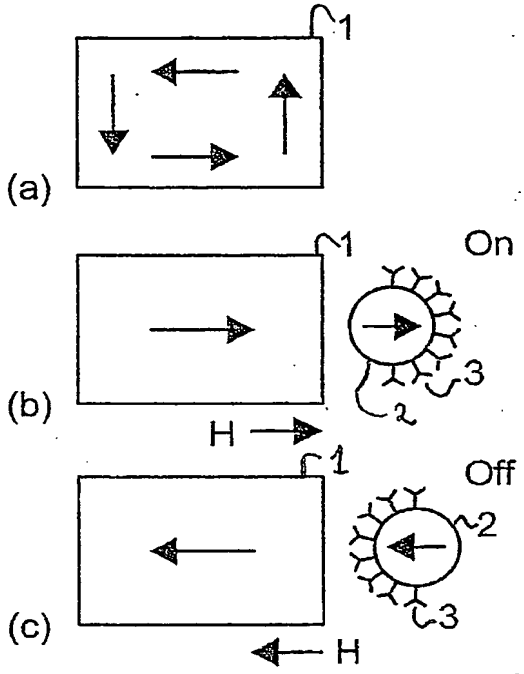


Fig. 1

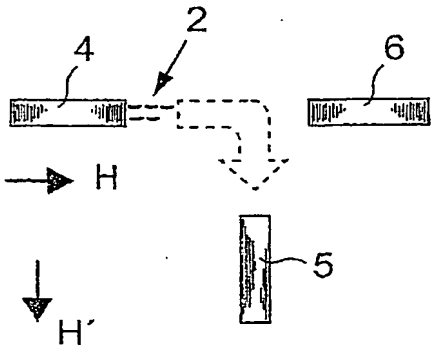


Fig. 2

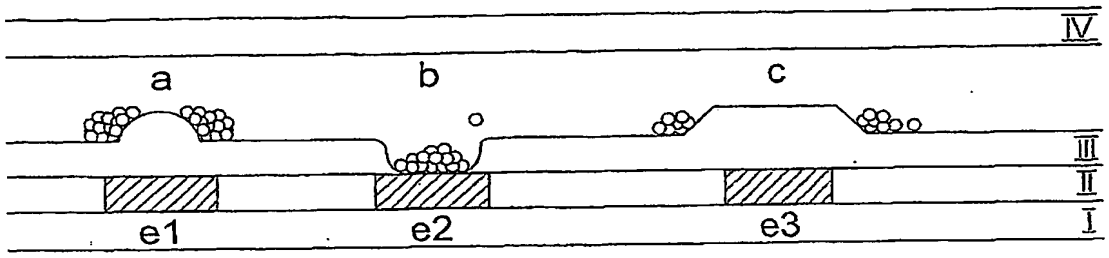


Fig. 3

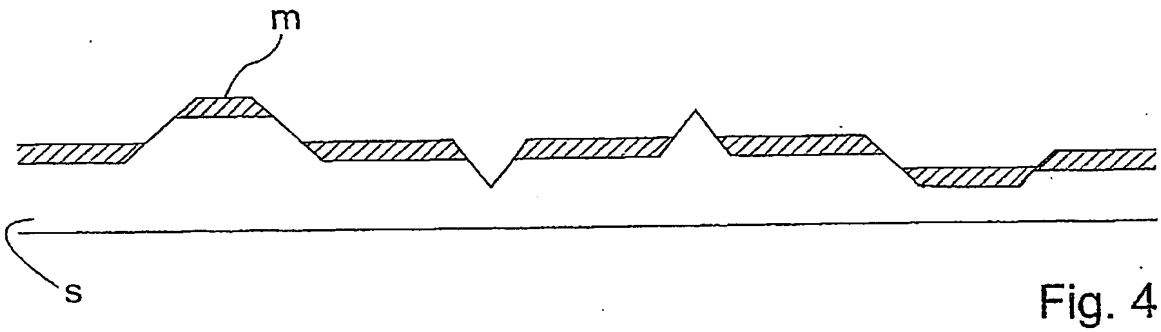


Fig. 4

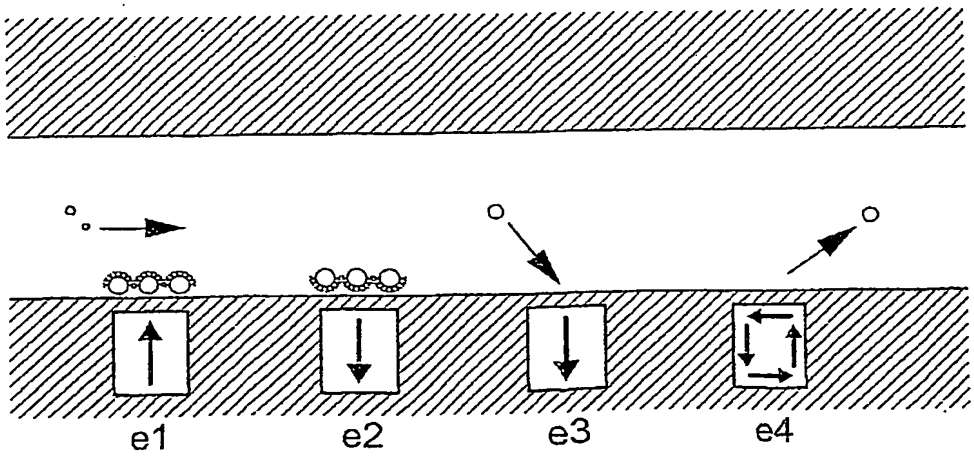


Fig. 5

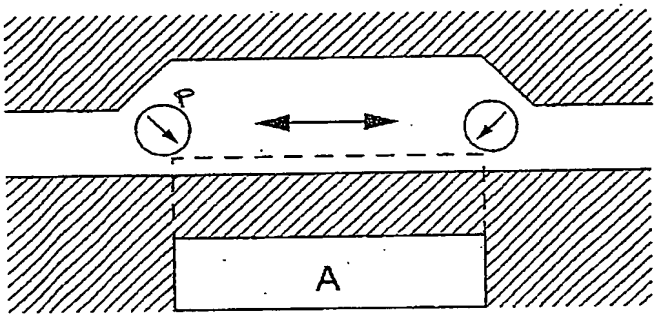


Fig. 6a

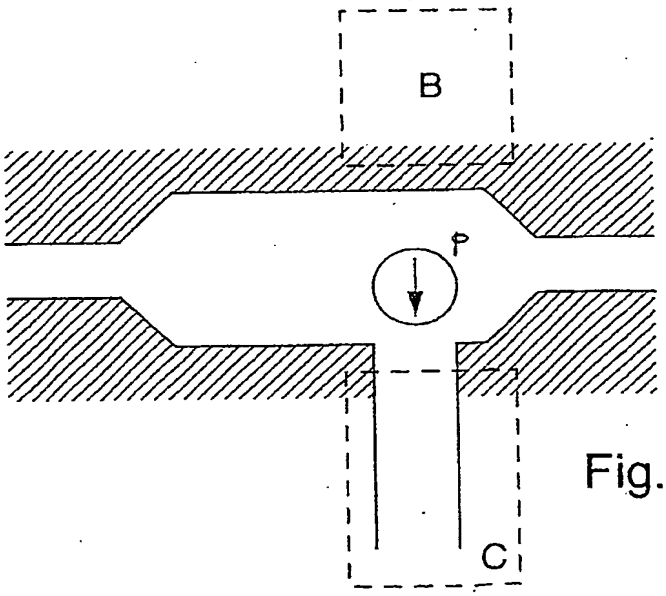


Fig. 6b

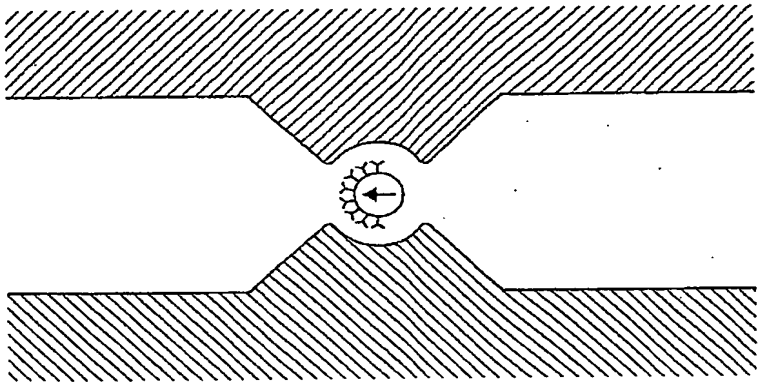


Fig. 7a

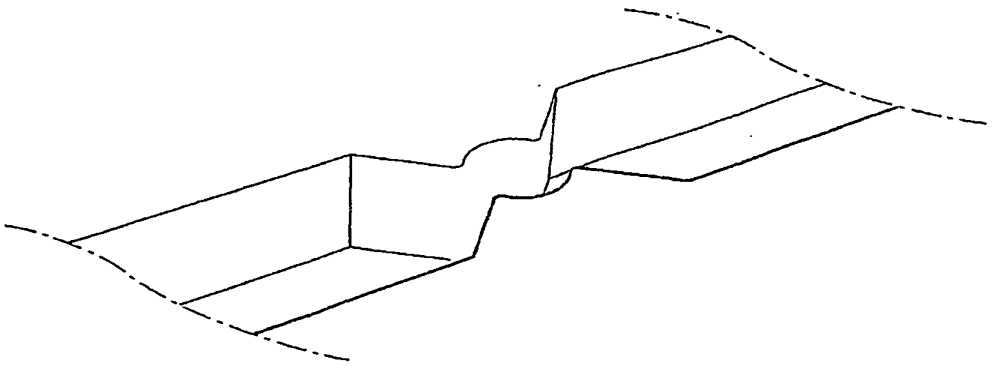


Fig. 7b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/02099

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B81B 7/04, B82B 1/00, G01N 33/543, F16K 31/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B81B, B82B, G01N, B25J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, EPO-INTERNAL, PAJ, COMPENDEX, INSPEC, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0054882 A1 (ARTLOON CORPORATION ET AL), 21 Sept 2000 (21.09.00), page 6, line 10 - line 14; page 7, line 5 - page 12, line 15; page 14, line 1 - page 17, line 14, page 23, line 1 - page 24, line 4, page 39, line 6 - line 9, page 41, line 18 - line 20, figures 1-23	1-7,9-17, 19-20,22
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E,X	WO 02097422 A1 (ELECTRON-BIO, INC.), 5 December 2002 (05.12.02), page 4, line 5 - line 14; page 4, line 25 - page 5, line 13, figures 1A-B	21
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 2003	27 -02- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Jens Waltin/ELY Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

Information on patent family members

30/12/02

International application No.
PCT/SE 02/02099

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CN 1267089 A	20/09/00
		EP 1207959 A	29/05/02
		US 6355491 B	12/03/02
		US 2002022276 A	21/02/02
WO 02097422 A1	05/12/02	NONE	
WO 0157506 A1	09/08/01	AU 6751400 A	14/08/01
		US 6468809 B	22/10/02
		US 2002197739 A	26/12/02
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		EP 0920627 A	09/06/99
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		US 6136182 A	24/10/00
WO 0212896 A1	14/02/02	AU 7492200 A	18/02/02
		CN 1337580 A	27/02/02

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/02099

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0157506 A1 (THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE NAVY), 9 August 2001 (09.08.01), page 2, line 34 - page 3, line 20; page 5; page 16, line 10 - line 16, page 17, line 6 - page 18, line 9, figures 1,14A, 15	1-7,9-11, 14-15,19
X	WO 9746882 A1 (IMMUNIVEST CORPORATION), 11 December 1997 (11.12.97), page 1, line 1 - line 14; page 12, line 5 - page 13, line 30; page 15, line 17 - line 27, page 16, line 31 - page 17, line 26	1-7,10-11, 15-17,19
P,X	WO 0212896 A1 (AVIVA BIOSCIENCES CORPORATION), 14 February 2002 (14.02.02), page 4, line 12 - page 5, line 15; page 9, line 14 - line 29; page 24, line 6, page 35, line 16 - page 36, line 31, page 42, line 1 - page 43, line 12, page 47, line 3 - page 48, line 3	1-7,9-11, 14-15,19
A	DATABASE WPI Week 200148 Derwent Publications Ltd., London, GB; Class B04, AN 2001-445832 & JP 2001132861 A (EBARA CORP), 18 May 2001 (2001-05-18) figures 1-7, abstract	21